Bladder and Kidney Cancer Following Cyclophosphamide Therapy for Non-Hodgkin's Lymphoma

*Lois B. Travis, Rochelle E. Curtis, Bengt Glimelius, Eric J. Holowaty, Flora E. Van Leeuwen, Charles F. Lynch, Anton Hagenbeek, Marilyn Stovall, Peter M. Banks, Johanna Adami, Mary K. Gospodarowicz, Sholom Wacholder, Peter D. Inskip, Margaret A. Tucker, John D. Boice, Jr.**

Background: **Cyclophosphamide is an established bladder carcinogen, but few studies have examined the relationship between dose and effect. The largest analysis to date included only seven cases of bladder cancer. No investigation has estimated the risk of kidney cancer.** ***Purpose:*** **The purpose of this study was to quantify the risk of bladder and kidney cancer following cyclophosphamide therapy.** ***Methods:*** **Within a cohort of 6171 two-year survivors of non-Hodgkin's lymphoma (NHL), 48 patients with secondary cancer of the urinary tract were identified and matched to 136 control subjects with NHL who did not develop a second malignancy. Detailed information on chemotherapeutic drugs and cumulative dose received was collected for all subjects. Radiation dose to the target organ was estimated from individual radiotherapy records. Evaluations of the risk of second cancer as a result of treatment with cyclophosphamide alone, radiation alone, or both therapies were made relative to those patients who were exposed to neither treatment modality.** ***Results:*** **A significant 4.5-fold risk of bladder cancer (95% confidence interval [CI] = 1.5-13.6) followed therapy with cyclophosphamide, and risk was dependent upon cumulative dose. Among patients who received a total amount of cyclophosphamide of less than 20 g, a nonsignificant 2.4-fold risk of bladder cancer was apparent. Significantly elevated sixfold (95% CI = 1.3-29) and 14.5-fold (95% CI = 2.3-94) risks of bladder malignancy followed cumulative doses of 20-49 g and 50 g or more, respectively (*P* value for trend = .004). Radiotherapy given without cyclophosphamide was associated with a nonsignificant increased risk of bladder malignancy. Excess bladder cancer risk following treatment with both radiotherapy and cyclophosphamide was as expected if individual risks were summed. Neither radiotherapy nor cyclophosphamide was associated with excesses of kidney cancer.** ***Conclusions:*** **Cyclophosphamide-related bladder cancer is dose dependent. For patients given cumulative doses between 20 and 49 g, the absolute risk of bladder cancer is on the order of three excess cancers per 100 NHL patients after 15 years of follow-up. At cumulative doses of 50 g or more, the excess risk increases to approximately seven excess bladder cancers per 100 NHL patients.** ***Implications:*** **The strong dose–response relationship and high absolute risk of bladder cancer underscore the importance of limiting the cumulative dose of cyclophosphamide to what is required to achieve therapeutic end points. The risk of secondary bladder malignancy and other late sequelae of therapy must be carefully weighed against the curative gains provided by cyclophosphamide. Moreover, long-term side effects of therapy that might be acceptable in cancer treatment may need to be re-evaluated for patients with non-neoplastic disorders. [J Natl Cancer Inst 87:524-530, 1995]**

Although the first case report linking cyclophosphamide with the development of bladder cancer appeared more than 20 years ago (*1*), the precise level of risk from this exposure is not known. Only two analytic studies (*2,3*) have evaluated bladder malignancy following cyclophosphamide treatment, and neither described a dose–response relationship. The largest study (*3*) to date provided an overall risk estimate based on only seven cases of cyclophosphamide-related bladder cancer; no investigation has estimated the risk of secondary kidney cancer. Since cyclophosphamide is now utilized to treat both neoplastic and non-neoplastic disease in 500 000 patients worldwide each year (*4*), further assessment of its long-term carcinogenic potential is important.

In an earlier study (*5*), we reported significantly increased risks of bladder and kidney cancer among 6171 two-year sur-

**Affiliations of authors:* L. B. Travis, R. E. Curtis, S. Wacholder, P. D. Inskip, M. A. Tucker, J. D. Boice, Jr., Epidemiology and Biostatistics Program, Division of Cancer Etiology, National Cancer Institute, Bethesda, Md.

B. Glimelius, J. Adami, University Hospital, Uppsala, Sweden.

E. J. Holowaty, The Ontario Cancer Treatment and Research Foundation, Toronto, Canada.

F. E. Van Leeuwen, The Netherlands Cancer Institute, Amsterdam.

C. F. Lynch, The University of Iowa, Iowa City.

A. Hagenbeek, The Dr. Daniel den Hoed Cancer Center, Rotterdam, The Netherlands.

M. Stovall, The University of Texas M. D. Anderson Cancer Center, Houston.

P. M. Banks, University of Texas Health Sciences Center, San Antonio.

M. K. Gospodarowicz, Princess Margaret Hospital, Toronto.

Correspondence to: Lois B. Travis, M.D., National Institutes of Health, Executive Plaza North, Suite 408, Bethesda, MD 20892.

See "Notes" section following "References."

vivors of non-Hodgkin's lymphoma (NHL) compared with the general population ($P<.05$). Since cyclophosphamide is widely used in the therapy of NHL, nested case–control studies were undertaken within this group of patients to explore the relationship of secondary urinary tract cancers to prior treatment. Detailed radiotherapy data were collected to evaluate any interaction between radiation and cyclophosphamide in the development of these second malignancies.

Subjects and Methods

Study Subjects

Case–control studies of secondary cancers of the bladder and kidney were conducted among NHL patients identified by three regional cancer registries (the State Health Registry of Iowa, The Ontario Cancer Registry, and The Swedish Cancer Register) and the affiliated tumor registries of The Netherlands Cancer Institute and the Dr. Daniel den Hoed Cancer Center (5). Eligibility criteria for this cohort, as outlined previously, included the following: 1) a diagnosis of NHL as a first primary cancer from January 1, 1965, through December 31, 1980; 2) age between 18 and 70 years at the time of initial diagnosis of NHL; and 3) survival of 2 or more years without the development of a second invasive primary malignancy (5). In addition, subjects received initial management at selected major hospitals except in Sweden, where all patients who met study entry criteria were included. The final cohort consisted of 6171 NHL patients.

Review of pathology records for reported second urinary tract cancers confirmed 31 transitional cell carcinomas of the bladder and 17 renal cell carcinomas. No transitional cell carcinomas of the renal pelvis or ureter, which have been previously reported following cyclophosphamide therapy (*6-10*), were identified. For each secondary cancer, three control subjects were chosen by random sampling from the NHL cohort. Matching factors included cancer registry (or institute in The Netherlands), sex, age, race, calendar year of diagnosis of NHL, and length of follow-up at least as long as the interval between the case subject's diagnosis of NHL and secondary cancer.

Data Abstraction

Demographic and medical record data, including information on all NHL therapy during the matched time interval, were abstracted onto standardized forms for all case and control subjects. Data sources included hospitals providing initial treatment, local medical centers, radiotherapy facilities, and offices of private physicians. Information on drug dose and length of administration was collected for all alkylating agents, including cyclophosphamide. For other cytotoxic agents, abstracted data were limited to dates and duration of administration. For 94.6% of the patients (93.8% case subjects and 94.9% control subjects), information on cumulative dose of cyclophosphamide was available from all utilized sources. For the remaining subjects, dose was estimated on the basis of duration of therapy (two case subjects and four control subjects) or imputed from the median dose (one case subject and three control subjects).

Radiation dose to the target organ (bladder or kidney) was calculated utilizing detailed radiotherapy records for each patient. In regions outside the radiation beam, dose was calculated by measurement in water phantoms (*11*). Radiotherapy was primarily administered by megavoltage. Treatment fields typically included the abdomen or pelvis or both (53%), head and neck only (22%), chest and/or mantle (16%), or other sites (9%).

Information on tobacco use at the time of NHL diagnosis was collected as available from medical records. We attempted to examine the role of smoking; however, data were incomplete. Limited evidence suggested that tobacco was not a confounder in this study, and there is no reason to believe that cyclophosphamide-treated patients were more or less likely to smoke than subjects given other therapies.

Statistical Analysis

The relative risk (RR) of urinary tract cancer associated with cyclophosphamide or radiotherapy was estimated by comparing the exposure history of the case subject with that of individually matched control subjects, utilizing standard conditional logistic regression programs (*12,13*). Analyses were conducted separately for bladder cancer case and control subjects and for kidney cancer case and control subjects.

Subjects were considered exposed if they received cyclophosphamide for 1 month or more (*14,15*) or more than 0.5 Gy of radiation to the target organ (bladder or kidney) (*16*). To allow an adequate latent period for radiation carcinogenesis, we utilized only radiotherapy given more than 5 years prior to the diagnosis of either a second bladder or kidney cancer (or an equivalent date for control subjects) in the analysis (*16*). This represented approximately 81% and 90%, respectively, of all radiation dose administered to bladder cancer case and control subjects and 71% of all dose given to both kidney cancer case and control subjects.

Since cyclophosphamide and radiation are established bladder carcinogens, patients were divided into four mutually exclusive groups based on all NHL treatment: cyclophosphamide without radiotherapy, radiotherapy without cyclophosphamide, cyclophosphamide with radiotherapy, or neither cyclophosphamide nor radiotherapy. Evaluations of the risk of bladder or kidney cancer in patients treated with cyclophosphamide or radiation were made relative to the risk in patients who were exposed to neither treatment modality (the referent category). Likelihood ratio tests were performed to evaluate differences between treatment groups. Two-sided P values and 95% confidence intervals (CIs) were calculated for all RRs.

The risk between dose (or duration) of cyclophosphamide and bladder cancer was estimated by dividing the cumulative dose (or duration) into interval categories and calculating the RR between each interval category and the referent group of patients exposed to neither cyclophosphamide nor radiation. Tests for trend were conducted by assigning the midpoint of the dose or duration of therapy as the representative score for that category (*17*).

The excess risk (excess number of cases) of bladder cancer among 100 NHL patients followed for 15 years was estimated by first multiplying the RR minus 1 by the expected number of bladder cancers per 100 person-years of follow-up, as estimated from our prior cohort study of NHL patients (0.053 bladder cancer/100 person-years) (5). The product was then multiplied by 10, which is the number of years at risk, assuming a latent period of 5 years before the occurrence of treatment-related bladder cancer (*5,18,19*). For example, a 10-fold risk of bladder cancer associated with cyclophosphamide would correspond to an excess of about five cases of bladder cancer per 100 patients over a 15-year period: $(10 - 1) \times 0.053 \times 10 = 4.77$.

Results

Characteristics of NHL patients with bladder (n = 31) or kidney (n = 17) cancer and individually matched controls are summarized in Table 1. The median age at diagnosis of NHL was 58 years for patients with secondary bladder cancer and 53 years for patients with secondary kidney cancer. The median latency between diagnoses of NHL and bladder or kidney cancer was 8.5 years (range, 3-21 years) and 9.0 years (range, 2-18 years), respectively.

Bladder Cancer

Cyclophosphamide was administered to 58% of NHL patients with secondary bladder cancer, compared with 34% of the matched control subjects (Table 1). Radiotherapy alone (no cyclophosphamide) was administered to 19% of case subjects and 18% of control subjects. The cumulative amount of cyclophosphamide and the radiation dose to the bladder are presented by treatment group in Table 2. Among subjects treated with radiotherapy without cyclophosphamide, the median radiation dose to the bladder was greater for control (20.4 Gy) than case subjects (12.9 Gy). No control subjects within this group received chemotherapy, although one case subject received chlorambucil.

The median cumulative dose of cyclophosphamide among NHL patients who did not receive radiotherapy was ap-

Table 1. Distribution of NHL patients with secondary bladder or kidney cancer (case subjects) and matched control subjects by selected characteristics

Characteristic	Site of cancer			
	Bladder		Kidney	
	Cases (n = 31)	Controls (n = 89)	Cases (n = 17)	Controls (n = 47)
Country				
United States (Iowa)	3	8	1	3
The Netherlands	9	26	1	3
Canada (Ontario)	12	36	4	12
Sweden	7	19	11	29
Sex				
Male	22	62	11	32
Female	9	27	6	15
Age at diagnosis of NHL, y				
<50	5	16	6	16
50-59	12	34	4	13
60-70	14	39	7	18
NHL stage				
I or II	16	40	8	27
III or IV	9	21	6	13
Unknown	6	28	3	7
Latency*, y				
2-4	2	7	4	11
5-9	17	46	6	18
≥10	12	36	7	18
NHL treatment, all†				
Radiotherapy, no cyclophosphamide	6	16	3	7
Cyclophosphamide, no radiotherapy	9	20	7	17
Cyclophosphamide, radiotherapy	9	10	1	5
Other‡	6	42	6	18

*Interval between diagnosis of NHL and bladder or kidney cancer for case subjects and comparable interval for matched control subjects.

†Exposure was defined as treatment with cyclophosphamide for more than 1 month or radiotherapy that resulted in a dose of 0.5 Gy or more to the organ of interest (bladder or kidney). Cyclophosphamide was usually given in combination with other drugs. Excluded from the list are one bladder case subject and one bladder control subject for whom radiotherapy status was unknown.

‡Includes all patients who received a radiation dose of <0.5 Gy to the organ of interest (bladder or kidney) and/or cyclophosphamide for less than 1 month.

proximately threefold greater for the nine case subjects than for the 20 control subjects within this category (36.8 g and 11.8 g, respectively). Cyclophosphamide was typically given in combination chemotherapy regimens with vincristine and prednisone (CVP) (*20*) (two case subjects and two control subjects); doxorubicin, vincristine, and prednisone (CHOP) (*21*) (three control subjects); vincristine, procarbazine, and prednisone (COPP) (*22*) (one case subject and three control subjects); and bleomycin, doxorubicin, vincristine, and prednisone (BACOP) (*23*) (one case subject and one control subject). Four case subjects and eight control subjects received more than one of the above cyclophosphamide-containing regimens, while one case

Table 2. Radiation dose to bladder and cumulative amount of cyclophosphamide administered to NHL patients with secondary bladder cancer (case) and matched controls by treatment group

	Treatment group*							
	Radiotherapy, no cyclophosphamide (n = 6 cases and 16 controls); radiation dose, Gy		Cyclophosphamide, no radiotherapy (n = 9 cases and 20 controls); cyclophosphamide dose, g		Cyclophosphamide, radiotherapy (n = 9 cases and 10 controls)			
					Cyclophosphamide dose, g		Radiation dose, Gy	
	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
Mean	15.5	21.0	51.4	26.1	25.1	14.8	23.4	14.8
Median	12.9	20.4	36.8	11.8	13.5	12.7	23.8	16.2
Range	0.9-38.8	1.2-58.7	8.8-146	3.7-176	6-60	2.1-46	1.9-38.8	2.6-32

*Treatment groups are mutually exclusive and reflect all therapy administered for NHL within the matched time interval at risk. Exposure was defined as treatment with cyclophosphamide for more than 1 month or radiotherapy that resulted in a dose of 0.5 Gy or more to the bladder. Cyclophosphamide was ususally given in combination with other drugs (*see* "Results" section). Excluded from the table are six case subjects and 42 control subjects who did not meet exposure criteria and one case subject and one control subject for whom radiotherapy status was unknown.

subject and three control subjects received therapy with single-agent cyclophosphamide. Other drugs given to these patients included chlorambucil (two case subjects and four control subjects), carmustine (two control subjects), and melphalan (one control subject).

Among subjects given both cyclophosphamide and radiotherapy, the median doses of cyclophosphamide were approximately equivalent, with larger mean doses among case subjects than control subjects (25.1 g and 14.8 g, respectively). Within this treatment group, cyclophosphamide was given in combination chemotherapy regimens, such as CVP (three case subjects and three control subjects), CHOP (one case subject and two control subjects), COPP (one case subject), and BACOP (three control subjects). One case subject and one control subject received more than one of the above cyclophosphamide-containing regimens, while three case subjects and one control subject received therapy with single-agent cyclophosphamide. Other drugs included chlorambucil (four control subjects), etoposide (one control subject), and cisplatin (one control subject). The median radiation dose to the bladder was greater for case (23.8 Gy) than for control (16.2 Gy) subjects. Radiotherapy preceded cyclophosphamide in seven of the nine case subjects treated with combined modality therapy.

Radiotherapy given without cyclophosphamide was associated with a nonsignificant 2.8-fold risk of secondary bladder cancer compared with subjects whose treatment included neither radiation nor cyclophosphamide (Table 3). A dose–response relationship between radiation and bladder cancer risk was not apparent within this treatment group (P = .42). Cyclophosphamide therapy was associated with a significantly elevated 4.5-fold risk of bladder cancer (95% CI = 1.5-13.6). The route of administration (intravenously or by mouth) of cyclophosphamide was not related to subsequent excesses of bladder cancer (P = .37). Treatment with other cytotoxic agents or prednisone did not contribute to bladder cancer risk (P = .73 and .43, respectively), when cyclophosphamide was taken into account.

Table 4 shows the RR of bladder malignancy by categories of cumulative dose of cyclophosphamide or duration of therapy. A nonsignificant 2.4-fold risk of bladder cancer was evident among patients who received a cumulative dose of less than 20 g of cyclophosphamide. Significantly elevated sixfold and 14.5-fold risks of bladder cancer were noted among patients who received cumulative doses between 20 and 49 g and 50 g or more, respectively. Bladder cancer excesses increased with increasing cumulative dose of cyclophosphamide (P value for trend = .004). The duration of cyclophosphamide therapy was also strongly associated with the risk of bladder cancer. Patients who received less than 1 year of cyclophosphamide demonstrated a nonsignificant 2.5-fold risk of bladder cancer. The risk increased to 3.7-fold and 11.8-fold, respectively, among subjects who received between 1 and 2 years or 2 or more years of treatment, respectively (P value for trend = .003). Excesses of bladder cancer following cyclophosphamide did not differ by sex, age at diagnosis of NHL (<50 or ≥50 years), or study site. Radiation dose to the bladder among patients who received cyclophosphamide was significantly associated with bladder cancer risk (P = .01) when added to a model that adjusted for cumulative amount of cyclophosphamide.

Excesses of bladder malignancies in relation to both cyclophosphamide and radiotherapy were further examined by dividing dose of cyclophosphamide into two categories (<20 g and ≥20 g) and adjusting for the administration of radiation with a categorical (yes/no) variable (Table 5). Patients who received a cumulative dose of less than 20 g cyclophosphamide without radiotherapy had no demonstrable increased risk of bladder cancer (RR = 1.0; 95% CI = 0.2-4.3). A nonsignificant threefold risk was noted for subjects who received less than 20 g of cyclophosphamide accompanied by radiotherapy. Significantly increased risks of bladder cancer were apparent among patients given higher cumulative doses (≥20 g). Within this high-dose group, bladder cancer excesses varied according to the administration of radiotherapy, with eightfold risks apparent in patients whose treatment included both modalities, and fourfold risks among those who received cyclophosphamide alone.

Kidney Cancer

Among NHL patients included in the study of secondary kidney cancer, similar proportions (47%) of case and control subjects were treated with cyclophosphamide (Table 1). One case subject and five control subjects within this group also received radiotherapy. Cyclophosphamide was usually given as single-agent therapy (two case subjects and five control subjects) or in combination chemotherapy regimens such as COPP (two case subjects and seven control subjects), CVP (one case subject and three control subjects), BACOP (one control subject), and CHOP (one control subject). Combinations of the above regimens were administered to two case subjects and three con-

Table 3. Risk of bladder cancer according to all treatment administered for NHL*

Treatment group	No. of case subjects	No. of control subjects	Matched RR†	95% CI
No radiotherapy, no cyclophosphamide	6	42	1.0	—
Radiotherapy, no cyclophosphamide	6	16	2.8	0.7-11.1
Cyclophosphamide with or without radiotherapy	18	30	4.5‡	1.5-13.6
Other§	1	1	2.5	0.2-40

*Treatment groups are mutually exclusive and reflect all therapy administered for NHL within the matched time interval. Exposure was defined as treatment with cyclophosphamide for more than 1 month or radiotherapy that resulted in a dose of 0.5 Gy or more to the bladder. Cyclophosphamide was usually given in combination with other drugs.

†The referent group consists of six case subjects and 42 control subjects who did not meet exposure criteria (*see* Table 1, ‡ footnote).

‡P<.05.

§Includes one case subject and one control subject for whom radiotherapy status was unknown.

Table 4. Risk of bladder cancer according to cumulative dose and duration of cyclophosphamide therapy

Cyclophosphamide	Median dose or duration*	No. of cases	No. of controls	Matched RR†	95% CI
Cumulative dose, g					
<20‡	10.0 g	8	22	2.4	0.7-8.4
20-49	34.0 g	5	6	6.3§	1.3-29
≥50	87.7 g	5	2	14.5§,¶	2.3-94
Duration of therapy, y					
<1	6 mo	8	20	2.5	0.7-9.0
1-2	18 mo	3	6	3.7	0.6-22
≥2	51 mo	7	4	11.8§,¶	2.3-61

*Median cumulative dose of cyclophosphamide or median duration of therapy among all patients within the specified category.

†The referent group consists of six case subjects and 42 control subjects who did not meet exposure criteria (*see* Table 1, ‡ footnote). The multivariate model also included terms for patients who received radiotherapy without cyclophosphamide (six case subjects and 16 control subjects) or for whom radiotherapy status was unknown (one case subject and one control subject).

‡The minimum cumulative dose of cyclophosphamide in this group was 2.1 g.

§$P<.05$.

¶P for trend < .005.

Table 5. Risk of bladder cancer according to cumulative dose of cyclophosphamide and administration of radiotherapy

Cyclophosphamide category	Radiotherapy category: No, <0.5 Gy — Matched RR* (95% CI)	Radiotherapy category: Yes, ≥0.5 Gy — Matched RR* (95% CI)
<20 g	1.0 (0.2-4.3) (3 cases/14 controls)	3.3 (0.7-15) (5 cases/8 controls)
≥20 g	4.3† (1.1-16) (6 cases/6 controls)	8.1† (1.2-53) (4 cases/2 controls)

*The referent group consists of six case subjects and 42 control subjects who did not meet exposure criteria (*see* Table 1, ‡ footnote). The multivariate model also included terms for patients who received radiotherapy without cyclophosphamide (six case subjects and 16 control subjects) or for whom radiotherapy status was unknown (one case subject and one control subject).

†$P<.05$.

Table 6. Risk of kidney cancer according to all treatment administered for NHL*

Treatment group	No. of cases	No. of controls	Matched RR†	95% CI
No radiotherapy, no cyclophosphamide	6	18	1.0	—
Radiotherapy, no cyclophosphamide	3	7	1.3	0.2-7.7
Cyclophosphamide (all doses)	8	22	1.2	0.3-5.0
<20 g	3	16	0.4	0.1-2.9
≥20 g	5	6	2.6	0.5-13.7

*Treatment groups are mutually exclusive and reflect all therapy administered for NHL within the matched time interval. Exposure was defined as treatment with cyclophosphamide for more than 1 month or radiotherapy that resulted in a dose of 0.5 Gy or more to the kidney. Cyclophosphamide was usually given in combination with other drugs.

†The referent group consists of six case subjects and 18 control subjects who did not meet exposure criteria (*see* Table 1, ‡ footnote).

trol subjects, whereas other cyclophosphamide-containing combination chemotherapy was given to one case subject and two control subjects. Median cumulative doses of cyclophosphamide were higher for case subjects (20.8 g; range, 5.7-48 g) than for control subjects (11.0 g; range, 2.2-72 g). Other drugs administered to these patients included etoposide (one case subject and one control subject) and prednimustine (two case subjects and one control subject).

Among subjects treated with radiotherapy without cyclophosphamide, the median radiation dose to the kidney was greater for case subjects (12.8 Gy; range, 4.2-45 Gy) than for control subjects (4.8 Gy; range, 1.6-10.9 Gy). Other cytotoxic agents were not administered to patients in this treatment group.

Neither cyclophosphamide nor radiotherapy was associated with excesses of kidney cancer, with overall risks of 1.2 and 1.3, respectively (Table 6). Risk did not vary significantly by dose of cyclophosphamide, although NHL patients who received 20 g or more of cyclophosphamide demonstrated a 2.6-fold risk (P value for trend = .13). Evaluation of any interaction between radiation and cyclophosphamide was not possible, given the small number of patients who received both treatments.

Discussion

This study is the largest investigation of secondary bladder cancer following cyclophosphamide therapy that includes information on cumulative amount and quantifies the risk associated with successively increasing dose levels of this widely used therapeutic agent. In addition, this is one of the few studies to simultaneously evaluate the role of cyclophosphamide and radiation in bladder carcinogenesis. New observations include the quantification of bladder cancer risk over a wide range of cyclophosphamide doses and recognition of a possibly additive relationship with radiotherapy in the development of bladder malignancy.

Our overall 4.5-fold estimate of bladder cancer risk following cyclophosphamide is somewhat lower than the sevenfold to 10-fold risks found in other analytic series, which may reflect differences in cumulative dose (*2,3*). Kinlen (*2*) reported a 10-fold risk of bladder cancer (five cases observed) among 416 patients who received cyclophosphamide as immunosuppressive treatment for three or more months, although descriptions of dose or duration of therapy were not provided. In a study of secondary bladder cancer among 471 NHL patients treated with cyclophosphamide, the associated risk was approximately sevenfold (*3*). The high, narrow range of cumulative doses of cyclophosphamide (range, 83-129 g; median, 110 g) and the small number of bladder cancers (n = 7) in this series (*3*), however, precluded evaluation of a dose–response relationship.

Other than the risk estimates reported by Kinlen (*2*) and Pedersen-Bjergaard et al. (*3*), there are few analytic descriptions of the association between cyclophosphamide and bladder cancer that utilize modern statistical methods. The paucity of data is somewhat surprising, given the widespread use of cyclophosphamide, which is now administered to about half a million patients each year (*4*). The relationship between cyclophosphamide and bladder cancer typically has been documented by occasional case reports describing patients [a total of 34 accord-

ing to one review (*24*)] who received large cumulative doses (median, 164 g; range, 3-1100 g). Whether these results reflect biased reporting of bladder malignancy following high cumulative doses of cyclophosphamide but not low doses is unknown. Our study of bladder cancer, set within a large, well-defined cohort of NHL patients, provides important new information describing the risk of bladder malignancy associated with a spectrum of cyclophosphamide doses. Sixfold risks of bladder cancer followed cumulative amounts of 20-50 g, with RRs of 14.5 associated with doses of 50 g or more. Although bladder cancer excesses among subjects who received less than 20 g seemed confined to those individuals treated with radiotherapy, the small number of subjects in this subgroup analysis, with associated wide CIs for risk estimates, warrants cautious interpretation.

Bladder cancer following cyclophosphamide therapy does not appear related to drug-induced hemorrhagic cystitis (*3*), an observation that mitigates concern that evaluation of this side effect could lead to a spurious association between cyclophosphamide and bladder malignancy. Of course, it is possible, although unlikely, that some clinicians may request periodic urine cytologic evaluations for any patient being treated with cyclophosphamide. Any such practice, however, would primarily result in diagnosis of bladder malignancy at less invasive stages than in the general population, since cancers at this site are eventually detected; risk estimates of the relationship between cyclophosphamide and bladder cancer should not be grossly distorted.

The effects of combined cyclophosphamide and radiation exposure in the development of human bladder malignancy have not been previously described to our knowledge. Our results alert clinicians that the risk of treatment-induced bladder cancer may be greater when both cyclophosphamide and radiotherapy are employed than when only single modality therapy is given. An increased incidence of toxic effects to the bladder was previously noted among children with Hodgkin's disease or Ewing's sarcoma treated with both pelvic radiotherapy and cyclophosphamide (*25*). The number and severity of bladder-related complications among these patients prompted the authors to advise consideration of an alkylating agent other than cyclophosphamide for subjects who receive or will receive radiotherapy to the pelvic area (*25*). It is unlikely, however, that procedures now known to temper cyclophosphamide-induced bladder toxicity, such as intensive intravenous hydration or administration of sodium 2-mercaptoethane-sulfonate (*26*), were undertaken in this population (*25*).

Cyclophosphamide produces a general hyperplasia of both bladder epithelium and endothelium (*27*). Since radiation has typically been thought to act as an initiating agent in carcinogenesis, it has been speculated that cyclophosphamide-induced proliferation might contribute to an earlier expression of radiation injury (*28*). Recent evidence, however, suggests that acrolein, the major toxic metabolite of cyclophosphamide, initiates bladder carcinogenesis in the rat (*29*). Other preclinical studies (*28*) show that the increased severity of bladder damage following treatment with both cyclophosphamide and radiation is not strongly related to the sequence or time interval between the two agents. An ongoing molecular component of our investigation seeks to define the spectrum of genetic mutations within secondary bladder cancers in relationship to prior treatment with cyclophosphamide, with or without radiotherapy.

The magnitude of the nonsignificant 2.8-fold risk of bladder malignancy following radiotherapy without cyclophosphamide in our series is consistent with prior studies of cancer mortality among atomic bomb survivors (*30*) and women irradiated for benign gynecologic disorders (*31*). In the largest study (*32*) to date of radiation-associated bladder cancer, women who received high doses (30-60 Gy) to this organ during treatment for cervical cancer demonstrated a fourfold risk of secondary bladder malignancy.

Significantly increased risks for renal cell carcinoma following NHL have been noted in data reported to cancer registries participating in the Surveillance, Epidemiology, and End Results (SEER) Program[1] of the National Cancer Institute (NCI) (*19*) and in our international cohort study (*5*). In the present investigation, neither cyclophosphamide nor radiation was significantly associated with kidney cancer excesses, although numbers in our study may have been too small to allow detection of increased risks. Although the mechanisms for the elevated risks of renal cell carcinoma following NHL are not known, future studies should also evaluate the role of diagnostic surveillance during patient follow-up.

Our results should be interpreted within the context of several strengths and weaknesses of this international investigation. The strengths of our study include the near-complete ascertainment of chemotherapy and radiotherapy data and the substantial number of NHL patients (more than 6100 2-year survivors) included in the underlying cohort. A limitation is the relatively small number of cancers, 48 total, which restricts the inferences that can be drawn when subgroup analyses are undertaken. Nevertheless, our results clearly indicate that the risk of bladder cancer is closely related to cumulative amount of cyclophosphamide and is likely heightened by radiotherapy that results in a significant dose to the bladder.

Utilizing data from the underlying NHL population (*5*) together with the results of this study, it can be estimated that an excess of three bladder cancers might be expected among 100 NHL patients treated with cumulative doses of cyclophosphamide between 20 g and 50 g and followed for 15 years. At total amounts of 50 g or more, an excess of seven bladder cancers per 100 patients might result. As always, the risk of subsequent bladder malignancy and other late effects of cyclophosphamide must be weighed against the therapeutic gain achieved through its use. Risk–benefit issues vary for different diseases, particularly non-neoplastic disorders in which concern already exists that the toxicity of long-term cyclophosphamide might outweigh the available therapeutic benefit (*33*). Our quantification of one of the most serious late sequelae of cyclophosphamide therapy, i.e., bladder cancer, enables more rigorous assessment of this facet of the risk–benefit equation.

References

(*1*) Worth PH: Cyclophosphamide and the bladder. Br Med J 3:182, 1971

(*2*) Kinlen LJ: Incidence of cancer in rheumatoid arthritis and other disorders after immunosuppressive treatment. Am J Med 78:44-49, 1985

(3) Pedersen-Bjergaard J, Ersboll J, Hansen VL, et al: Carcinoma of the urinary bladder after treatment with cyclophosphamide for non-Hodgkin's lymphoma. N Engl J Med 318:1028-1032, 1988
(4) Fraiser LH, Kanekal S, Kehrer JP: Cyclophosphamide toxicity. Characterizing and avoiding the problem. Drugs 42:781-795, 1991
(5) Travis LB, Curtis RE, Glimelius B, et al: Second cancers among long-term survivors of non-Hodgkin's lymphoma. J Natl Cancer Inst 85:1932-1937, 1993
(6) Fuchs EF, Kay R, Poole R, et al: Uroepithelial carcinoma in association with cyclophosphamide ingestion. J Urol 126:544-545, 1981
(7) McDougal WS, Cramer SF, Miller R: Invasive carcinoma of the renal pelvis following cyclophosphamide therapy for nonmalignant disease. Cancer 48:691-695, 1981
(8) Schiff HI, Finkel M, Schapira HE: Transitional cell carcinoma of the ureter associated with cyclophosphamide therapy for benign disease: a case report. J Urol 128:1023-1024, 1982
(9) Brenner DW, Schellhammer PF: Upper tract urothelial malignancy after cyclophosphamide therapy: a case report and literature review. J Urol 137:1226-1227, 1987
(10) Levine E: Transitional cell carcinoma of the renal pelvis associated with cyclophosphamide therapy. AJR Am J Roentgenol 159:1027-1028, 1992
(11) Stovall M, Smith SA, Rosenstein M: Tissue doses from radiotherapy of cancer of the uterine cervix. Med Phys 16:726-733, 1989
(12) Breslow NE, Day NE: The analysis of case-control studies. *In* Statistical Methods in Cancer Research, vol. 1. IARC Sci Publ No. 32. Lyon, France: IARC, 1980
(13) Lubin JH: A computer program for the analysis of matched case-control studies. Comput Biomed Res 14:138-143, 1981
(14) Curtis RE, Boice JD Jr, Stovall M, et al: Risk of leukemia after chemotherapy and radiation treatment for breast cancer [*see* comment citations in Medline]. N Engl J Med 326:1745-1751, 1992
(15) Travis LB, Curtis RE, Stovall M, et al: Risk of leukemia following therapy for non-Hodgkin's lymphoma. J Natl Cancer Inst 86:1450-1457, 1994
(16) Committee on the Biologic Effects of Ionizing Radiations (BEIR V), National Research Council: Health Effects of Exposure to Low Levels of Ionizing Radiation. Washington, DC: Natl Acad Press, 1990
(17) Rothman KJ: Analysis with multiple levels of exposure. *In* Modern Epidemiology (Rothman KJ, ed). Boston: Little, Brown, 1986, pp 327-350
(18) Travis LB, Curtis RE, Boice JD Jr, et al: Bladder cancer following chemotherapy for non-Hodgkin's lymphoma [letter]. N Engl J Med 321:544-545, 1989
(19) Travis LB, Curtis RE, Boice JD Jr, et al: Second cancers following non-Hodgkin's lymphoma. Cancer 67:2002-2009, 1991
(20) Bagley CM Jr, DeVita VT Jr, Berard CW, et al: Advanced lymphosarcoma: intensive cyclical combination chemotherapy with cyclophosphamide, vincristine, and prednisone. Ann Intern Med 76:227-234, 1972
(21) McKelvey EM, Gottlieb JA, Wilson HE, et al: Hydroxyldaunomycin (Adriamycin) combination chemotherapy in malignant lymphoma. Cancer 38:1484-1493, 1976
(22) Stein RS, Moran EM, Desser RK, et al: Combination chemotherapy of lymphomas other than Hodgkin's disease. Ann Intern Med 81:601-609, 1974
(23) Schein PS, DeVita VT Jr, Hubbard S, et al: Bleomycin, Adriamycin, cyclophosphamide, vincristine, and prednisone (BACOP) combination chemotherapy in the treatment of advanced diffuse histiocytic lymphoma. Ann Intern Med 85:417-422, 1976
(24) Samra Y, Hertz M, Lindner A: Urinary bladder tumors following cyclophosphamide therapy: a report of two cases with a review of the literature. Med Pediatr Oncol 13:86-91, 1985
(25) Jayalakshmamma B, Pinkel D: Urinary-bladder toxicity following pelvic irradiation and simultaneous cyclophosphamide therapy. Cancer 38:701-707, 1976
(26) DeVries CR, Freiha FS: Hemorrhagic cystitis: a review. J Urol 143:1-7, 1990
(27) Koss LG: A light and electron microscopic study of the effects of a single dose of cyclophosphamide on various organs in the rat. I. The urinary bladder. Lab Invest 16:44-65, 1967
(28) Edrees G, Luts A, Stewart F: Bladder damage in mice after combined treatment with cyclophosphamide and X-rays. The influence of timing and sequence. Radiother Oncol 11:349-360, 1988
(29) Cohen SM, Garland EM, St. John M, et al: Acrolein initiates rat urinary bladder carcinogenesis. Cancer Res 52:3577-3581,1992
(30) Darby SC, Nakashima E, Kato H: A parallel analysis of cancer mortality among atomic bomb survivors and patients with ankylosing spondylitis given x-ray therapy. J Natl Cancer Inst 75:1-21, 1985
(31) Inskip PD, Monson RR, Wagoner JK, et al: Cancer mortality following radium treatment for uterine bleeding [published erratum appears in Radiat Res 128:326, 1991]. Radiat Res 123:331-344, 1990
(32) Boice JD Jr, Engholm G, Kleinerman RA, et al: Radiation dose and second cancer risk in patients treated for cancer of the cervix. Radiat Res 116:3-55, 1988
(33) Hoffman GS, Kerr GS, Leavitt RY, et al: Wegener granulomatosis: an analysis of 158 patients [*see* comment citations in Medline]. Ann Intern Med 116:488-498, 1992

Notes

[1]*Editor's note:* SEER is a set of geographically defined, population-based central tumor registries in the United States, operated by local nonprofit organizations under contract to the NCI. Each registry annually submits its cases to the NCI on a computer tape. These computer tapes are then edited by the NCI and made available for analysis.

We are indebted to the many hospitals and physicians who allowed access to treatment records; to Virginia Hunter, Loretta Brown, Sondra van den Belt, Robert Oppliger, and Nancy Holt for support in data collection; to Diana Wagner and Carla Van Hoesen for assistance in identifying cases; to Diane Fuchs of Westat, Inc. (Rockville, Md.) for administrative assistance in conducting the field studies; to Roberta McClimens and Dave Hacker from Information Management Services (Silver Spring, Md.) for computing support; and to Rebecca Albert for technical assistance.

Manuscript received August 24, 1994; revised December 7, 1994; accepted January 20, 1995.